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PROVISIONAL SPECIFICATION

FOR THE INVENTION ENTITLED:

"ORGAN ARREST PROTECTION AND PRESERVATION"

Applicant:

JAMES COOK UNIVERSITY

The invention is described in the following statement:

ORGAN ARREST, PROTECTION AND PRESERVATION

The present invention relates to a method and pharmaceutical or veterinary composition for arresting, protecting and/or preserving organs, in particular the heart during open-heart surgery, cardiovascular diagnosis or therapeutic intervention.

The heart may be arrested for up to 3 hours during open-heart surgery. High potassium cardioplegia (in excess of 15-20 mM) has been the basis of myocardial arrest and protection for over 40 years. Currently the majority of solutions used contain high potassium including the widely used St Thomas No. 2 Hospital Solution which generally contains 110 mM NaCl, 16 mM KCl, 16 mM $MgCl_2$, 1.2 mM $CaCl_2$ and 10 mM $NaHCO_3$ and has a pH of about 7.8. Notwithstanding hyperkalemic solutions providing acceptable clinical outcomes, recent evidence suggests that progressive potassium induced depolarisation leads to ionic and metabolic imbalances that may be linked to myocardial stunning, ventricular arrhythmias, ischaemic injury, cell swelling and loss of function during the reperfusion period. The major ion imbalances postulated are linked to an increased sodium influx which in turn activates the Na^+/Ca^{2+} exchangers leading to a rise in intracellular Ca^{2+}. Compensatory activation of Na^+ and Ca^{2+} ion pumps then occur, which activate anaerobic metabolism to replenish ATP with a concomitant increase in tissue lactate and fall in tissue pH. Free radical generation and oxidative stress have also been implicated in potassium arrest and partially reversed by the administration of antioxidants. In some cases, high potassium induced ischaemia has been reported to have damaged smooth muscle and endothelial function.

In an attempt to minimise ischaemic damage during cardioplegic arrest, an increasing number of studies have employed potassium channel openers instead of high potassium. Cardioprotection using nicorandil, aprikalim or pinacidil is believed to be linked to the opening of the potassium channel which leads to a hyperpolarised state, a shortening of the action potential and decreasing Ca^{2+} influx into the cell. One shortfall however is that the heart takes the same time or longer to recover than with high potassium cardioplegic solutions. Another limitation is

that pinacidil requires a carrier due to its decreased solubility in aqueous solutions. The carrier routinely used is dimethyl sulphoxide (DMSO) which is controversial when used in animal or human therapy.

Most investigators including those using potassium channel openers, believe that as soon as blood flow is halted, and the arrest solution administered, ischaemia occurs and progressively increases with time. In contrast, we sought a cardioplegic solution that would place the heart in a reversible hypometabolic state analogous to the tissues of a hibernating turtle, a hummingbird in torpor, an aestivating desert frog or a hibernating bear. When these animals drop their metabolic rate (some by over 90%), their tissues do not become progressively ischaemic but remain in a steady state where supply and demand are matched. An ideal cardioplegic solution should produce a readily reversible, rapid electrochemical arrest with minimal tissue ischaemia. The heart should accumulate little tissue lactate, utilise little glycogen, show minimal changes in high-energy phosphates, cytosolic redox (NAD/NADH) and the bioenergetic phosphorylation (ATP/ADP Pi) ratio and free energy of ATP. There should be little or no change in cytosolic pH or free magnesium, minimal water shifts between the intracellular and extracellular phases, and no major ultrastructural damage to organelles such as the mitochondria. The ideal cardioplegic solution should produce 100% functional recovery with no ventricular arrhythmia, cytosolic calcium overload or other pump abnormalities. There is no cardioplegic solution currently available which fulfils all these requirements. We have now found that the heart can be better protected during arrest and recovery by using the potassium channel opener adenosine and the local anaesthetic lignocaine.

The action of adenosine is controversial. Adenosine has been shown to increase coronary blood flow, hyperpolarise the cell membrane and act as a preconditioning agent via the ATP-sensitive potassium channel and adenosine related pathways including adenosine receptors notably the A1 receptor. Adenosine is also known to improve myocardial recovery as an adjunct to high potassium cardioplegia. Furthermore, adenosine can be used as a pretreatment (whether or not it is present in the arresting solution) to reduce lethal injury. In one study, adenosine was shown to rival potassium arrest solutions and more recently in blood

cardioplegia, it prevented post-ischaemic dysfunction in ischaemically injured hearts. Adenosine is sometimes added as an adjunct to potassium cardioplegia.

Lignocaine is a known local anaesthetic which blocks sodium fast channels and has antiarrhythmatic properties by reducing the magnitude of inward sodium current. The accompanying shortening of the action potential is thought to directly reduce calcium entry into the cell via Ca^{2+} selective channels and Na^{+}/Ca^{2+} exchange. Recent reports also implicate lignocaine with the scavenging of free radicals such as hydroxyl and singlet oxygen in the heart during reperfusion. Associated with this scavenging function, lignocaine may also inhibit phospholipase activity and minimise membrane degradation during ischaemia. Lignocaine has also been shown to have a myocardial protective effect and in one study was found to be superior to high potassium solutions. However, our experiments show that lignocaine alone at 0.5, 1.0 and 1.5 mM gave highly variable functional recoveries.

According to one aspect of the present invention there is provided a method for arresting, protecting and/or preserving an organ which includes administering effective amounts of (i) a potassium channel opener or agonist and/or an adenosine receptor agonist and (ii) a local anaesthetic to a subject in need thereof.

According to another aspect of the present invention there is provided the use of (i) a potassium channel opener or agonist and/or an adenosine receptor agonist and (ii) a local anaesthetic in the manufacture of a medicament for arresting, protecting and/or preserving an organ.

The present invention also provides (i) a potassium channel opener or agonist and/or an adenosine receptor agonist and (ii) a local anaesthetic for use in arresting, protecting and/or preserving an organ.

According to a further aspect of the present invention there is provided a pharmaceutical or veterinary composition which includes effective amounts of (i) a potassium channel opener or agonist and/or an adenosine receptor agonist and (ii) a local anaesthetic.

While the present invention is particularly advantageous in arresting, protecting and/or preserving an organ while it is intact in the body of the subject, it

will be appreciated that it may also be used to arrest, protect and/or preserve isolated organs.

Thus, the present invention still further provides a method for arresting, protecting and/or preserving an organ which includes adding a composition which includes effective amounts of (i) a potassium channel opener or agonist and/or an adenosine receptor agonist and (ii) a local anaesthetic to the organ.

The term "adding" is used herein in its broadest sense to refer to any methods of exposing the organ to the composition of the present invention, for example, bathing, perfusing or pumping via various routes.

The term "organ" is used herein in its broadest sense and refers to any part of the body exercising a specific function including tissues and cells or parts thereof, for example, cell lines or organelle preparations. Other examples include circulatory organs such as the heart, respiratory organs such as the lungs, urinary organs such as the kidneys or bladder, digestive organs such as the stomach, liver, pancreas or spleen, reproductive organs such as the scrotum, testis, ovaries or uterus, neurological organs such as the brain, germ cells such as spermatozoa or ovum and somatic cells such as skin cells, heart cells i.e., myocytes, nerve cells, brain cells or kidney cells.

The method of the present invention is particularly useful in arresting, protecting and/or preserving the heart during open-heart surgery. Other applications include reducing heart damage before, during or following cardiovascular intervention which may include a heart attack, angioplasty or angiography. For example, the composition could be administered to subjects who have suffered or are developing a heart attack and used at the time of administration of blood clot-busting drugs such as streptokinase. As the clot is dissolved, the presence of the composition may protect the heart from further injury such as reperfusion injury. The composition may be particularly effective as a cardioprotectant in those portions of the heart that have been starved of normal flow, nutrients and/or oxygen for different periods of time. For example, the composition may be used to treat heart ischaemia which could be pre-existing or induced by cardiovascular intervention.

Thus, the present invention also provides a cardioplegic or cardioprotectant composition which includes effective amounts of (i) a potassium channel opener or agonist and/or an adenosine receptor agonist and (ii) a local anaesthetic.

The potassium channel openers or agonists may be selected from nicorandil, diazoxide, minoxidil, pinicadil, aprikalim, cromokulim, NS-1619 (1,3-dihydro-1-[2-hydroxy5(trifluoromethyl)phenyl]5-(trifluoromethyl)2-H-benimidazol-one), amlodipine, Bay K 8644(L-type)(1,4-dihydro-26-dimethyl-5-nitro-4[2(trifluoromethyl)phenyl]-3-pyridine carboxylic acid (methyl ester)), bepridil HCl (L-type), calciseptine (L-type), omega-conotoxin GVIA (N-type), omega-conotoxin MVIIC (Q-type), cyproheptadine HCl, dantrolene sodium (Ca^{2+} release inhibitor), diltiazem HCl (L-type), filodipine, flunarizine HCl (Ca^{2+}/Na^{+}), fluspirilene (L-type), HA-1077 2HCl(1-(5 isoquinolinyl sulphonyl) homo piperazine.HCl), isradipine, loperamide HCl, manoalide (Ca^{2+} release inhibitor), nicardipine HCl (L-type), nifedipine (L-type), niguldipine HCl (L-type), nimodipine (L-type), nitrendipine (L-type), pimozide (L- and T- type), ruthenium red, ryanodine (SR channels), taicatoxin, verapamil HCl (L-type), methoxy-verapamil HCl (L-type), YS-035 HCl (L-type)N[2(3,4-dimethoxyphenyl)ethyl]-3,4-dimethoxy N-methyl benzene ethaneamine HCl) and AV blockers such as verapamil and adenosine. It will be appreciated that this list includes calcium antagonists as potassium channel openers are indirect calcium antagonists.

Adenosine is particularly preferred as it is capable of opening the potassium channel, hyperpolarising the cell, depressing metabolic function, possibly protecting endothelial cells, enhancing preconditioning of tissue and protecting from ischaemia or damage. Adenosine is also an indirect calcium antagonist, vasodilator, antiarrhythmic, antiadrenergic, free radical scavenger, arresting agent, anti-inflammatory agent (attenuates neutrophil activation), metabolic agent and possible nitric oxide donor.

In a preferred embodiment, the present invention provides a method for arresting, protecting and/or preserving an organ which includes administering effective amounts of adenosine and a local anaesthetic to a subject in need thereof.

Suitable adenosine receptor agonists include N^6-cyclopentyladenosine (CPA), N-ethylcarboxamido adenosine (NECA), 2-[p-(2-carboxyethyl)phenethyl-amino-5'-N-ethylcarboxamido adenosine (CGS-21680), 2-chloroadenosine, N^6-[2-(3,5-dimethoxyphenyl)-2-(2-methoxyphenyl]ethyladenosine, 2-chloro-N^6-cyclopentyladenosine (CCPA), N-(4-aminobenzyl)-9-[5-(methylcarbonyl)-beta-D-robofuranosyl]-adenine (AB-MECA), ([IS-[1a,2b,3b,4a(S*)]]-4-[7-[[2-(3-chloro-2-thienyl)-1-methyl-propyl]amino]-3H-imidazole[4,5-b]pyridyl-3-yl]cyclopentane carboxamide (AMP579), N^6-(R)-phenylisopropyladenosine (R-PLA), aminophenylethyladenosine 9APNEA) and cyclohexyladenosine (CHA).

The local anaesthetic can be selected from mexiletine, diphenylhydantoin or Class 1B antiarrhythmic agents such as lignocaine or derivatives thereof, for example, QX-314. Lignocaine is preferred as it is capable of acting as a local anaesthetic probably by blocking calcium fast channels, depressing metabolic function, lowering free cytosolic calcium, protecting against enzyme release from cells, possibly protecting endothelial cells and protecting against myofilament damage. Lignocaine is also a free radical scavenger and an antiarrhythmic.

Thus, in a particularly preferred embodiment there is provided a method for arresting, protecting and/or preserving an organ which includes administering effective amounts of adenosine and lignocaine to a subject in need thereof.

In another preferred embodiment there is provided a pharmaceutical or veterinary composition which includes effective amounts of adenosine and lignocaine.

For ease of reference, the "potassium channel opener or agonist and/or adenosine receptor agonist" and the "local anaesthetic" will hereinafter be referred to as the "active ingredients".

The method of the present invention involves the administration of effective amounts of the active ingredients for a time and under conditions sufficient for the organ to be arrested, protected and/or preserved. The active ingredients may be administered separately, sequentially or simultaneously and in a single dose or series of doses.

The subject may be a human or an animal such as a livestock animal (e.g. sheep, cow or horse), laboratory test animal (e.g. mouse, rabbit or guinea pig) or a companion animal (e.g. dog or cat), particularly an animal of economic importance.

It will be appreciated that the amounts of active ingredients present in the composition will depend on the nature of the subject, the type of organ being arrested, protected and/or preserved and the proposed application. In the case of a human subject requiring heart arrest during open-heart surgery, the concentration of adenosine is preferably about 0.001 to about 20mM, more preferably about 0.01 to about 10mM, most preferably about 0.05 to about 5mM and the concentration of lignocaine is preferably about 0.001 to about 20mM, more preferably about 0.01 to about 10mM, most preferably about 0.05 to about 5mM. In the case of a human subject requiring treatment before, during or following a heart attack or cardiovascular intervention, the preferred concentrations of adenosine and lignocaine are set out in the table below.

Site of Injection	Type/Units	Adenosine	Lignocaine
Intravenous	Infusion	1. 0.001-10	1. 0.0001-20
	mg/min/kg	2. 0.01-5	2. 0.01-10
		3. 0.1-1	3. 0.5-3
Intravenous	Bolus	1. 0.0001-100	1. 0.001-1000
	mg/kg	2. 0.001-10	2. 0.01-100
		3. 0.005-1.0	3. 0.1-10
Intracoronary	Infusion	1. 0.0001-100	1. 0.005-50
	mg/min	2. 0.001-1	2. 0.005-5
	(per heart)	3. 0.01-0.5	3. 0.05-2.5
Intracoronary	Bolus	1. 0.001-1000	1. 0.01-10,000
	µg	2. 0.1-100	2. 1-1000
	(per heart)	3. 1-20	3. 10-200

1 = preferably
2 = more preferably
3 = most preferably

The active ingredients may be administered by any suitable route, including oral, implant, rectal, inhalation or insufflation (through the mouth or nose), topical (including buccal and sublingual), vaginal and parenteral (including subcutaneous, intramuscular, intravenous, intrasternal and intradermal). Preferably, administration in open-heart surgery or cardiovascular intervention applications will be achieved by mixing the active ingredients with the blood of the subject or subjects having a similar blood type. The active ingredients then enter the coronary circulation generally via the aorta. Heart arrest may also be achieved by either continuous or intermittent perfusion retrograde through the aorta in the Langendorff mode. However, it will be appreciated that the preferred route will vary with the condition and age of the subject and the chosen active ingredients.

While it is possible for one or both of the active ingredients to be administered alone, it is preferable to administer one or both of them together with one or more pharmaceutically acceptable carriers, diluents adjuvants and/or excipients. Each carrier, diluent, adjuvant and/or excipient must be pharmaceutically "acceptable" in the sense of being compatible with the other ingredients of the composition and not injurious to the subject. The compositions may conveniently be presented in unit dosage form and may be prepared by methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. Preferably, the compositions are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers, diluents, adjuvants and/or excipients.

The present invention also extends to a pharmaceutical or veterinary composition which includes the active ingredients and a pharmaceutically or veterinarily acceptable carrier, diluent, adjuvant and/or excipient.

Compositions of the present invention suitable for oral administration may be presented as discrete units such as capsules, sachets or tablets each containing a predetermined amount of the active ingredients; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water

liquid emulsion or a water-in-oil liquid emulsion. The active ingredients may also be presented as a bolus, electuary or paste.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder (e.g. pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methyl cellulose), fillers (e.g. lactose, microcyrstalline cellulose or calcium hydrogen phosphate), lubricants (e.g. magnesium stearate, talc or silica), inert diluent, preservative, disintegrant (e.g. magnesium stearate, talc or silica), inert diluent, preservative, disintegrant (e.g. sodium starch glycollate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agents. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile. Tablets may optionally be provided with an enteric coating, to provide release in parts of the gut other than the stomach.

Liquid preparations for administration prior to arresting, protecting and/or preserving the organ may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g. sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g. lecithin or acacia); non-aqueous vehicles (e.g. almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); preservatives (e.g. methyl or propyl-p-hydroxybenzoates or sorbic acid); and energy sources (e.g. carbohydrates such as glucose, fats such as palmitate or amino acid).

Compositions suitable for topical administration in the mouth include lozenges comprising the active ingredients in a flavoured basis, usually sucrose and

acacia or tragacanth gum; pastilles comprising the active ingredients in an inert basis such as gelatin and glycerin, or sucrose and acacia gum; and mouthwashes comprising the active ingredients in a suitable liquid carrier.

For topical application for the skin, the active ingredients may be in the form of a cream, ointment, jelly, solution or suspension.

For topical application to the eye, the active ingredients may be in the form of a solution or suspension in a suitable sterile aqueous or non-aqueous vehicle. Additives, for instance buffers, preservatives including bactericidal and fungicidal agents, such as phenyl mercuric acetate or nitrate, benzalkonium chloride or chlorohexidine and thickening agents such as hypromellose may also be included.

The active ingredients may also be formulated as depot preparations. Such long acting formulations may be administered by implantation (e.g. subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the active ingredients may be formulated with suitable polymeric or hydrophobic materials (e.g. as an emulsion in an acceptable oil or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

Compositions for rectal administration may be presented as a supposity or retention enema with a suitable non-irritation excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the active ingredients. Such excipients include cocoa butter or a salicylate.

For intranasal and pulmonary administration, the active ingredients may be formulated as solutions or suspensions for administration via a suitable metered or unit dose device or alternatively as a powder mix with a suitable carrier for administration using a suitable delivery device.

Compositions suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Compositions suitable for parenteral administration include aqueous and non-aqueous isotonic sterile injection solutions which may contain anti-oxidants,

buffers, bacteriostats and solutes which render the composition isotonic with the blood of the intended subject; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The compositions may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described

When the composition is for verterinary use it may be prepared, for example, by methods that are conventional in the art. Examples of such veterinary compositions include those adapted for:

(a) oral administration, external application, for example drenches (e.g. aqueous or non-aqueous solutions or suspensions); tablets or boluses; powders, granules or pellets for admixture with feedstuffs; pastes for application to the tongue;

(b) parenteral administration for example by subcutaneous, intramuscular or intravenous injection, e.g. as a sterile solution or suspension; or (when appropriate) by intramammary injection where a suspension or solution is introduced into the udder via the teat;

(c) topical application, e.g. as a cream, ointment or spray applied to the skin; or

(d) intravaginally, e.g. as a pessary, cream or foam.

It should be understood that in addition to the ingredients particularly mentioned above, the compositions of this invention may include other agents conventional in the art having regard to the type of composition in question, for example, those suitable for oral administration may include such further agents as binders, sweeteners, thickeners, flavouring agents, disintegrating agents, coating agents, preservatives, lubricants and/or time delay agents.

Suitable sweeteners include sucrose, lactose, glucose, aspartame or saccharin. Suitable disintegrating agents include corn starch, methylcellulose, polyvinylpyrrolidone, xanthan gum, bentonite, alginic acid or agar. Suitable

flavouring agents include peppermint oil, oil of wintergreen, cherry, orange or raspberry flavouring. Suitable coating agents include polymers or copolymers of acrylic acid and/or methacrylic acid and/or their esters, waxes, fatty alcohols, zein, shellac or gluten. Suitable preservatives include sodium benzoate, vitamin E, alpha-tocopherol, ascorbic acid, methyl paraben, propyl paraben or sodium bisulphite. Suitable lubricants include magnesium stearate, steric acid, sodium oleate, sodium chloride or talc. Suitable time delay agents include glyceryl monostearate or glyceryl distearate.

A preferred pharmaceutically acceptable carrier is a buffer having a pH of about 6 to about 9, preferably about 7, more preferably about 7.4 and/or low concentrations of potassium, for example, up to about 10mM, more preferably about 2 to about 8 mM, most preferably about 4 to about 6mM. Suitable buffers include Krebs-Henseleit which generally contains 10mM glucose, 117 mM NaCl, 5.9 mM KCl, 25 mM $NaHCO_3$, 1.2 mM NaH_2PO_4, 1.12 mM$CaCl_2$ (free Ca^{2+}=1.07mM), 0.512 mM $MgCl_2$ (free Mg^{2+}=0.5mM) and 1.2mM P, St. Thomas No. 2 solution, Tyrodes solution which generally contains 10mM glucose, 126 mM NaCl, 5.4 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 0.33 mM NaH_2PO_4 and 10 mM HEPES (N-[2-hydroxyethyl]piperazine-N'-[2-ethane sulphonic acid], Fremes solution, Hartmanns solution which generally contains 129 NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 29 mM lactate and Ringers-Lactate. One advantage of using low potassium is that it renders the present composition less injurous to the subject, in particular pediatric subjects. High potassium has been linked to an accumulation of calcium which may be associated with irregular heart beats during recovery, heart damage and cell swelling. Infants are even more susceptible than adults to high potassium damage during cardiac arrest. After surgery for defects an infant's heart may not return to normal for many days, sometimes requiring intensive therapy or life support. It is also advantageous to use carriers having low concentrations of magnesium, such as, for example up to about 2.5mM, but it will be appreciated that high concentrations of magnesium, for example up to about 20mM, can be used if desired without substantially effecting the activity of the composition.

In a further preferred embodiment the present invention provides a pharmaceutical or veterinary composition which includes adenosine, lignocaine and a pharmaceutically acceptable carrier which contains up to about 10mM potassium.

In a still further preferred embodiment, the present invention provides a pharmaceutical or veterinary composition which includes adenosine, lignocaine and Krebs-Henseleit buffer.

The composition may also advantageously be presented in the form of a kit in which the active ingredients are held separately for separate, sequential or simultaneous administration.

It will be appreciated that the composition of the present invention may also include and/or be used in combination with known medicaments depending on the proposed application. For instance, medicaments which substantially prevent the breakdown of adenosine in the blood such as nucleoside transport inhibitors, for example, dipyridamole could be used as additives in the composition of the present invention. The half life of adenosine in the blood is about 10 seconds so the presence of a medicament to substantially prevent its breakdown will maximise the effect of the composition of the present invention. Dipyridamole could advantageously be included in concentrations from about 0.1nM to about 10 mM and has major advantages with respect to cardioprotection. Dipyridamole may supplement the actions of adenosine by inhibiting adenosine transport which increases vasodilation. This could be particularly important when the composition is administered intermittently.

The invention will now be described with reference to the following examples. These examples are not to be construed as limiting in any way.

In the example, reference will be made to the accompanying drawing in which:

Figure 1 is a graph of aortic flow *vs* time comparing hearts arrested using 100μM adenosine and 0.5 mM lignocaine in Krebs-Henseleit and St. Thomas Hospital No. 2 solution;

Figure 2 is four graphs showing 20min ischaemia in rat heart *in vivo* following coronary artery ligation with no adenosine-lignocaine infusion;

Figure 3 is four graphs showing 20min ischaemia in rat heart *in vivo* following coronary artery ligation when infused with adenosine (6.3mg/ml) and lignocaine (12.6mg/ml) at 1ml/hour/300g rat ;

Figure 4 is a graph showing 30min ischaemia in rat heart *in vivo* following coronary artery ligation when infused with adenosine (6.3mg/ml) and lignocaine (12.6mg/ml) at 1ml/hour/300g rat;

Figure 5 is four graphs showing 20min ischaemia in rat heart *in vivo* following coronary artery ligation when infused with adenosine (3.15mg/ml) and lignocaine (12.6mg/ml) at 1ml/hour/300g rat;

Figure 6 is four graphs showing 30min ischaemia in rat heart *in vivo* following coronary artery ligation when infused with adenosine (1.6mg/ml) and lignocaine (12.6mg/ml) at 1ml/hour/300g rat; and

Figure 7 is a graph showing 30min ischaemia in rat heart *in vivo* following coronary artery ligation when infused with adenosine (1.6mg/ml) and lignocaine (12.6mg/ml) at 1ml/hour/300g rat.

EXAMPLE 1

This example compares the effects of adenosine (100μM) cardioplegia with hyperkalemic St. Thomas Hospital No. 2 solution (16 mM K^+) on functional recovery after a period of global ischaemia.

Hearts from male 450g Sprague-Dawley rats (n=19) were perfused for 30 minutes in the working mode (preload 7.5 mmHg; afterload 100 mmHg) with Krebs-Henseleit pH 7.4 buffer at 37°C. Hearts were then arrested in a retrograde mode at a constant pressure of 70 mmHg with either (i) a solution containing 100 μM adenosine and 0.5 mM lignocaine in filtered Krebs-Henseleit (10 mM glucose, pH 7.6 – 7.8 @ 37°C) (n=11) or (ii) St. Thomas No 2 solution (0.2 micron filter) (n=8). Following 30 minutes of arrest, the hearts were switched back to normal antegrade perfusion with Krebs-Henseleit pH 7.4 @ 37°C. Heart rate, coronary flow, aortic flow, aortic pressure and oxygen consumption were monitored every 10 minutes for 30 minutes. Statistical significance was assessed using a Student t-Test.

Results

Hearts arrested using adenosine cardioplegia achieved quiescence in half the time compared to St. Thomas No. 2 solution (30 *vs* 75 seconds, $p<0.0001$). During arrest under a constant perfusion pressure, coronary blood flow was 30% greater using adenosine cardioplegia ($p<0.05$). Adenosine arrested hearts also recovered faster (89±10 *vs* 119±30 seconds), but because of the greater variability in St. Thomas group the difference was not significant. Recovery of all functional parameters at 5 minutes was significantly better in the adenosine group ($p<0.05$); aortic flow (24 *vs* 9 ml/min), coronary flow (14 *vs* 9 ml/min), O_2 consumption (5.0 *vs* 2.3 µmol/min/g wet wt) and heart rate (213 *vs* 113 bpm). Over the next 30 minutes all measured functional parameters in each group were not significantly different. The results are summarised in Table 1 below and shown in Figure 1.

Table 1

	Adenosine and Lignocaine	**St. Thomas No. 2 Solution**	
Time to electrical and mechanical arrest	30 sec (n=11)	75 sec (n=8)	($p<0.0001$)
5 Minute recovery			
Heart rate (bpm)	213	113	($p<0.05$)
Aortic flow (ml/min)	24	9.1	($p<0.05$)
Coronary flow (ml/min)	14	9.2	($p<0.05$)
MVO_2 (µmol/min/g wet wt)	5.0	2.3	($p<0.05$)

In terms of functional parameters, 100µM adenosine and 0.5 mM lignocaine cardioplegia lead to shorter arrest times and an enhanced recovery profile compared to the St. Thomas Hospital No. 2 solution.

EXAMPLE 2

Male Wistar rats (250g) were housed in a temperature and light-controlled room. Food and water were provided freely until the day before the experiment

when the food was withheld and the rats were fasted overnight. The rats were anaesthetised with an intraperitoneal injection of pentobarbital (60mg kg^{-1}). Under anaesthesia, the rats were implanted with cannulas in the femoral vein and artery for adenosine and lignocaine (AL) administration and blood pressure measurement, respectively. A tracheotomy was performed and the rats were artificially ventilated with room air at 60 to 70 breaths/min. The chests of the rats were cut open and the left anterior descending (LAD) coronary artery located. A piece of suture was placed underneath LAD. After a 20min baseline period, LAD of the group of experimental rats were ligated for 30min and blood pressure and heart rate monitored. After 30 min of ischaemia, the ligature was released and the heart reperfused for 20 min. In the control rats, no AL was administered as shown in Figure 2. In the AL infusion 3 rats were used at three different doses of adenosine:

(1) 6.3 mg/ml adenosine + 12.6 mg/ml lignocaine infused at 1 ml/hr/300 g rat as shown in Figures 3 and 4;

(2) 3.15 mg/ml adenosine + 12.6 mg/ml lignocaine infused at 1 ml/hr/300 g rat as shown in Figure 5; and

(3) 1.6 mg/ml adenosine + 12.6 mg/ml lignocaine infused at 1 ml/hr/300 g rat as shown in Figures 6 and 7.

Compared to rats with 30 min ischaemia (no AL infusion) it was found that AL protected the heart in a dose dependent manner with the greatest protection occurring at the higher doses. As the dose of adenosine was halved, the protection was progressively lost. However, even in the worse case, the function of the heart was significantly better than with no AL alone. All hearts in rats receiving AL recovered in rate and pressure.

EXAMPLE 3

Adult Wistar rats (350g) were prepared using the method described in Example 2 and then subject to either continuous or intermittent perfusion as discussed below.

Continuous Perfusion

The mode of delivery of cardioplegia was continuous under a constant pressure head of 70mmHg after hearts were switched back from the working mode

tot he Langendorff mode. After different times of arrest up to 4hrs at 37°C the heart was switched back to the working mode under the normal oxygenated conditions described above. The results are shown in Table 2 below.

Table 2

4.0 hr of AL Constant Perfusion Cardioplegia

	Heart Rate (bpm)	Aortic Press (mmHg)	Aortic Flow (ml/min)	C/Flow (ml/min)
Control	262 ± 26	130/90	29 ± 3	15 ± 0.6
		4.00 hr hour arrest at 37°C		
Recovery (working heart pumping against 70mmHg afterload)				
15min	158 ± 17	118/82	7 ± 3	8 ± 1
30min	178 ± 43 (68%)	98/79 (75%)	3 ± 3 (10%)	7 ± 1 (47%)

High potassium (16mm) St.Thomas hearts struggle after 2hr. They do not recover after 3 or 4hr (n=4)

Intermittent Perfusion

Intermittent retrograde perfusion was performed under a constant pressure head of 70mmHg after hearts were switched back from the working mode to the Lagendorff mode. After a stabilisation, the hearts were arrested using 50ml of either adenosine plus lignocaine cardioplegia or St Thomas Hospital No 2 solution. The aorta was then cross-clamped and the heart left to sit arrested for 20min, after which the clamp was released and 2min of arrest solution delivered from a pressure head of 70 mmHg. The clamp was replaced and this procedure continued for up to 4hrs at 37°C. The results are shown in Table 3 below.

Table 3

4.0 hr of AL Intermittent Ischaemic Perfusion

Cardioplegia (2min Pulses every 20min over 4hr)

	Heart Rate (bpm)	Aortic Press (mmHg)	Aortic Flow (ml/min)	C/Flow (ml/min)
Control	275 ± 12	118/71	36 ± 2	16 ± 1

4.00 hr hour rat arrest with 20min aortic clamp at 37°C

Recovery (working heart pumping against 70mmHg afterload)

15min	230 ± 16	111/75	20 ± 4	14 ± 2
30min	240 ± 19	113/75	25 ± 3	12 ± 1
60min	249 ± 16 (91%)	112/74 (95-104%)	26 ± 3 (72%)	11 ± 1 (90%)

EXAMPLE 4

Neonatal rat hearts (using 50-70g 20 day old rats) were prepared using the intermittent perfusion technique described in Example 3 except the pressure head of delivery and afterload was reduced to 50mmHg. The results are shown in Table 4 below.

Table 4

	Adenosine/Lignocaine	St Thomas Hospital	p
Arrest Time (s)	18.57 ±3.72 (7)	65.71 ±12.71 (7)	<0.05
Time to First Contraction Following Reperfusion (s)	23.83 ±3.03 (7)	55.75 ±12.97 (4)	<0.05
Time to Recover 50mmHg Aortic flow (s)	165 ±29.48 (7)	270 ±83.5 (4)	ns
Percentage of Hearts to Survive Reperfusion	100 (7)	57*(4)	<0.05
Arrhythmia Occurrence (%)	14 (7)	25 (4)	ns

*Denotes Statistical Significance Using Students t-test.

Conclusions

With A-L significantly faster arrest times and faster time to contraction in recovery compared to St Thomas Hospital solution.

40% of neonatal/infant rat hears receiving St Thomas solution failed to recover after 2 hr of intermittent infusion.

100% of neonatal/infant rat hearts receiving adenosine plus lignocaine recovered pump function against a 50mmHg pressure head.

Since modifications within the spirit and scope of the invention may be readily effected by persons skilled in the art, it is to be understood that the invention is not limited to the particular embodiment described, by way of example, hereinabove.

Dated: 23 November 1999

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Patent Attorneys for the Applicant:

JAMES COOK UNIVERSITY

Figure 1

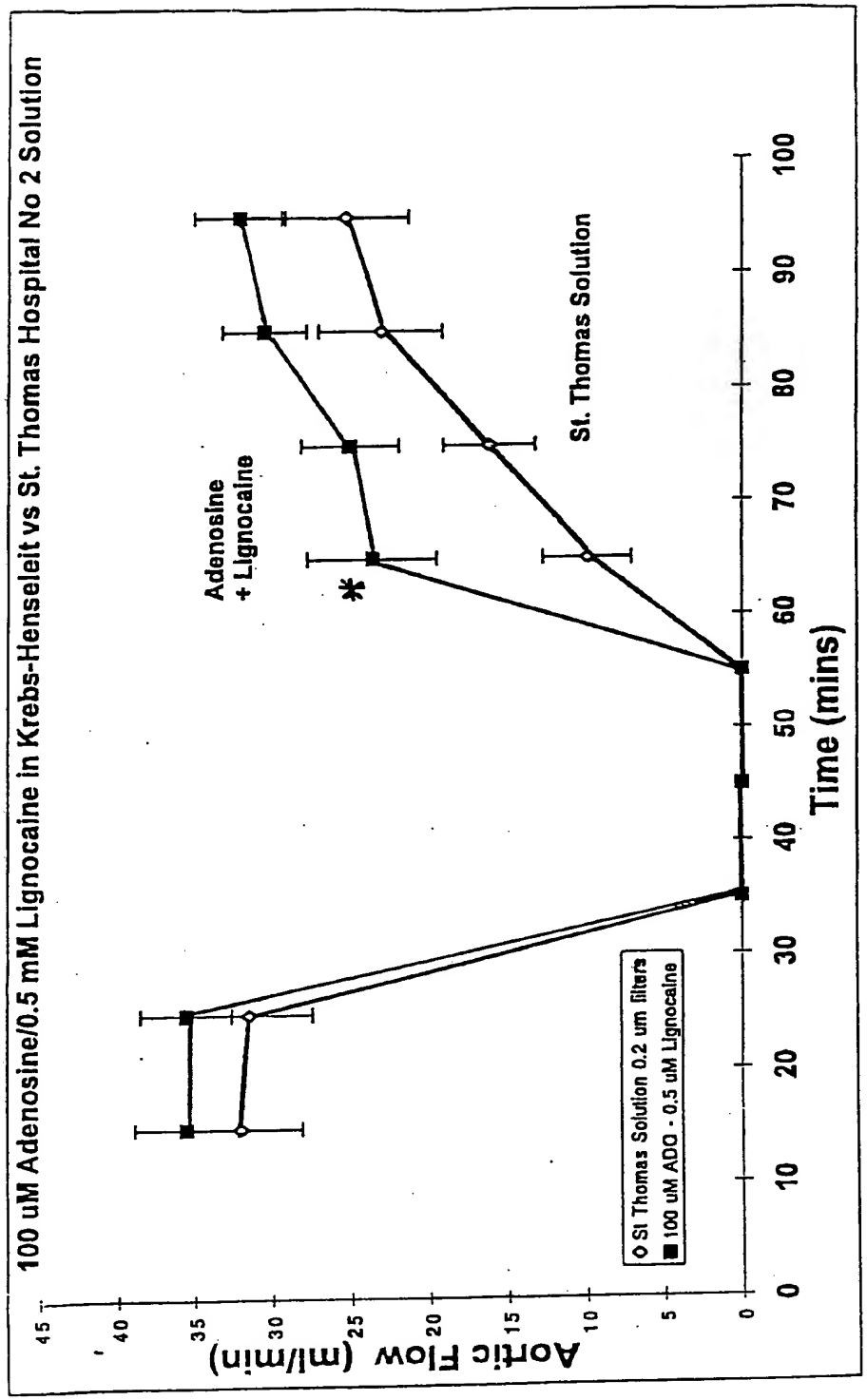

100 uM Adenosine/0.5 mM Lignocaine in Krebs-Henseleit vs St. Thomas Hospital No 2 Solution
Aortic Flow (ml/min)
Time (mins)
Adenosine
+ Lignocaine
St. Thomas Solution
*
St Thomas Solution 0.2 um filters
100 uM ADO - 0.5 uM Lignocaine
0
5
10
15
20
25
30
35
40
45
0
10
20
30
40
50
60
70
80
90
100

Figure 2

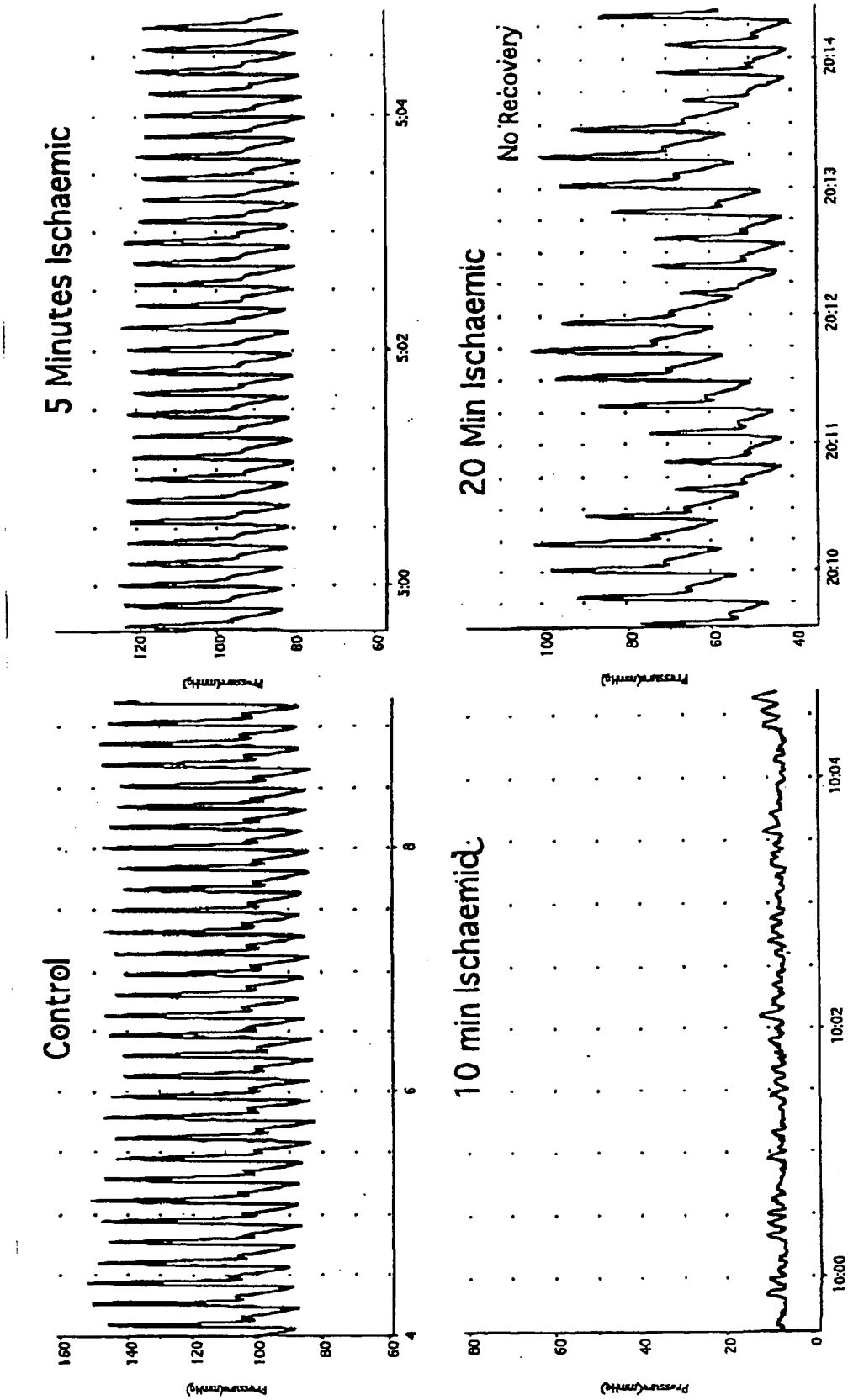

5 Minutes Ischaemic
Control
20 Min Ischaemic
No Recovery
10 min Ischaemic

Figure 3

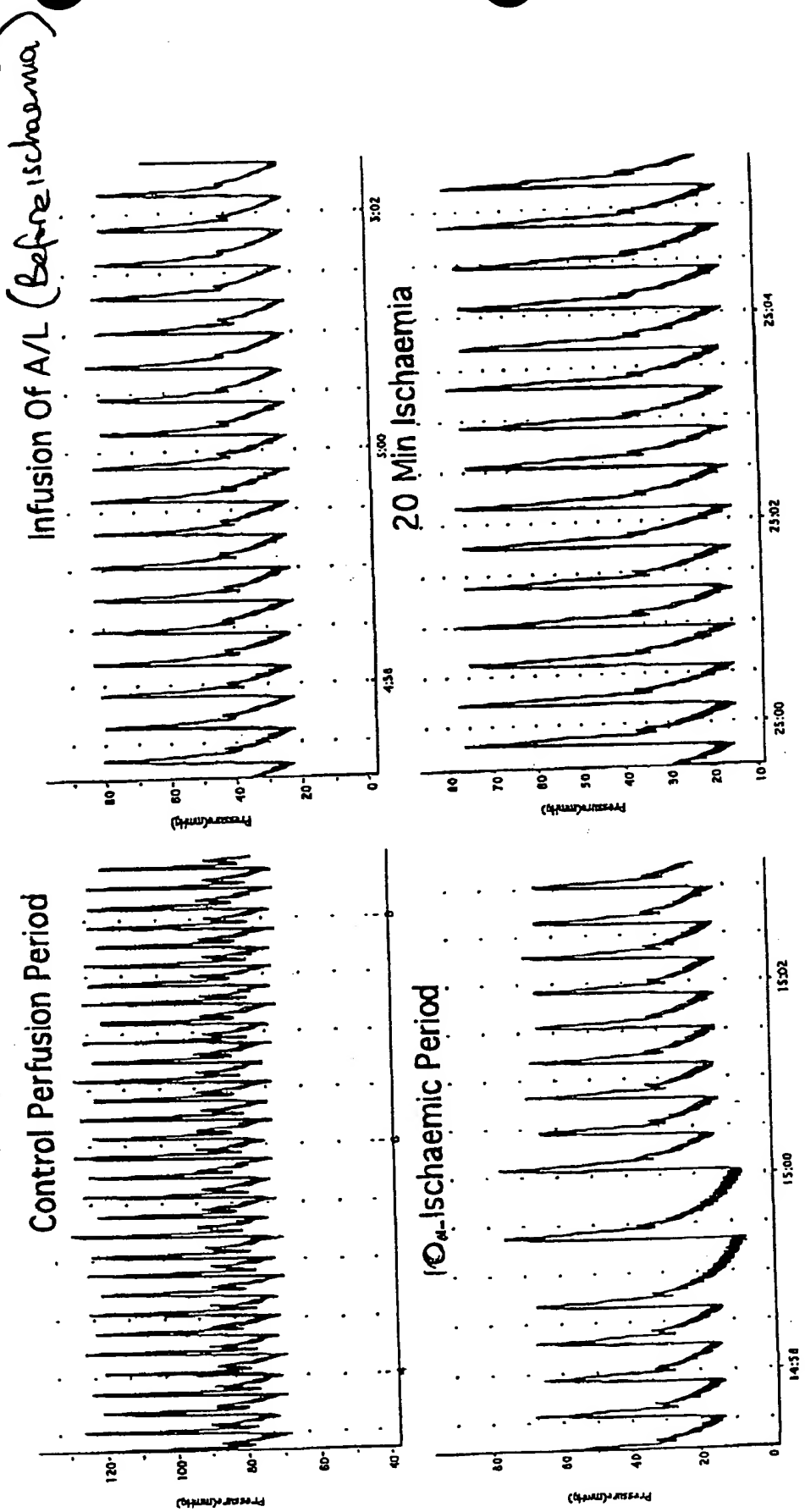

Infusion Of A/L (Before Ischaemia)
20 Min Ischaemia
Control Perfusion Period
$(O_{2d}$ Ischaemic Period

Figure 4

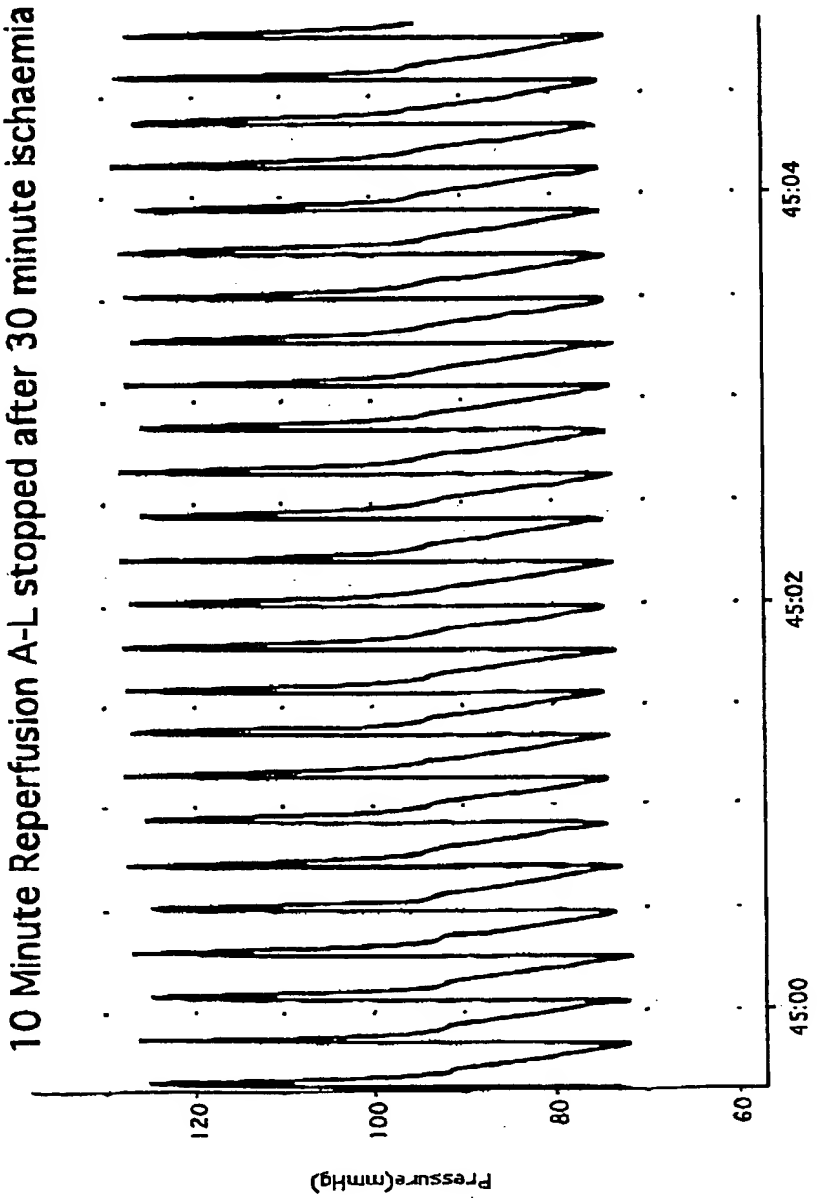

10 Minute Reperfusion A-L stopped after 30 minute ischaemia
Pressure(mmHg)
120
100
80
60
45:00
45:02
45:04

Figure 5

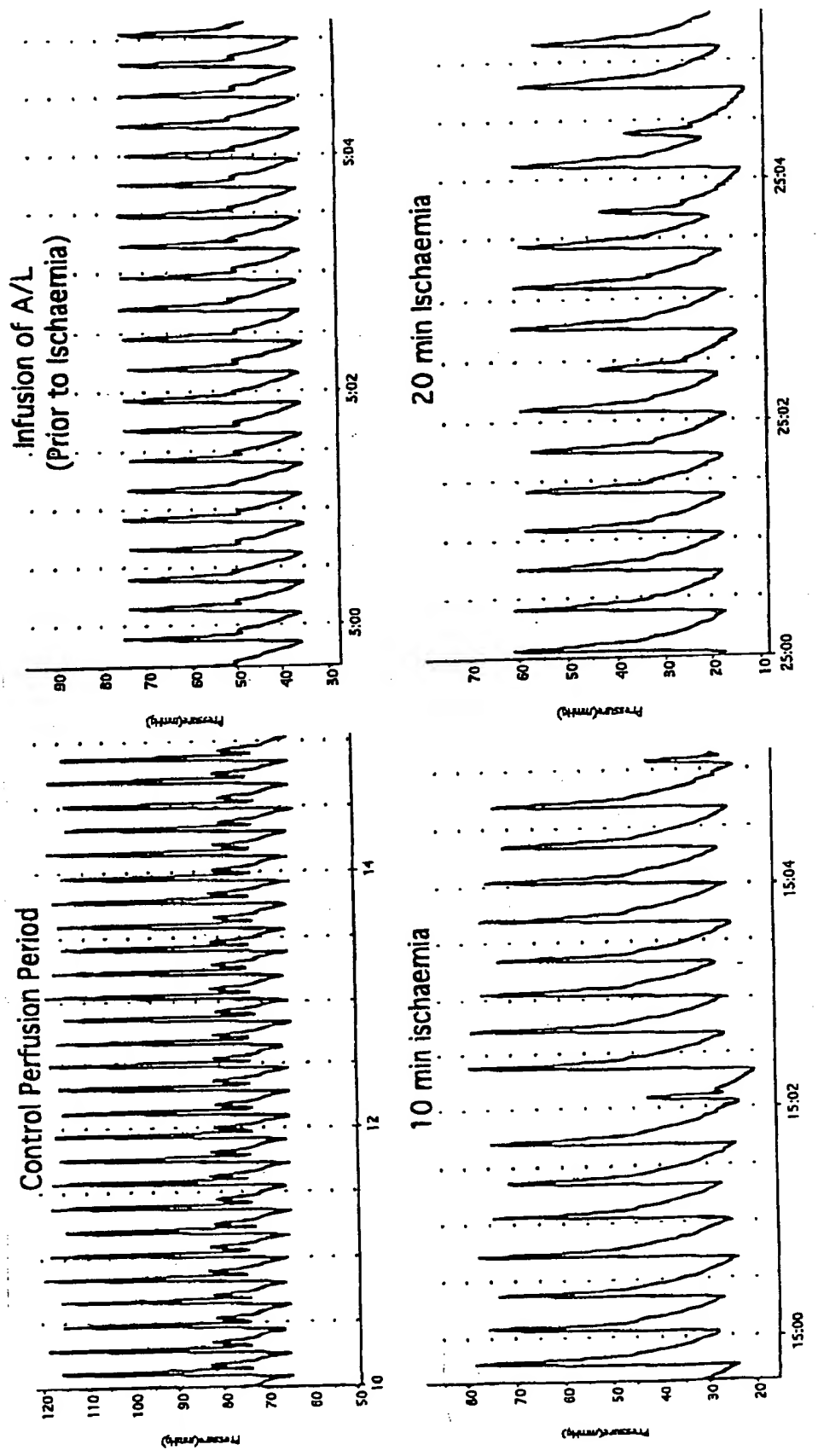

Infusion of A/L
(Prior to Ischaemia)
20 min Ischaemia
Control Perfusion Period
10 min ischaemia

Figure 6

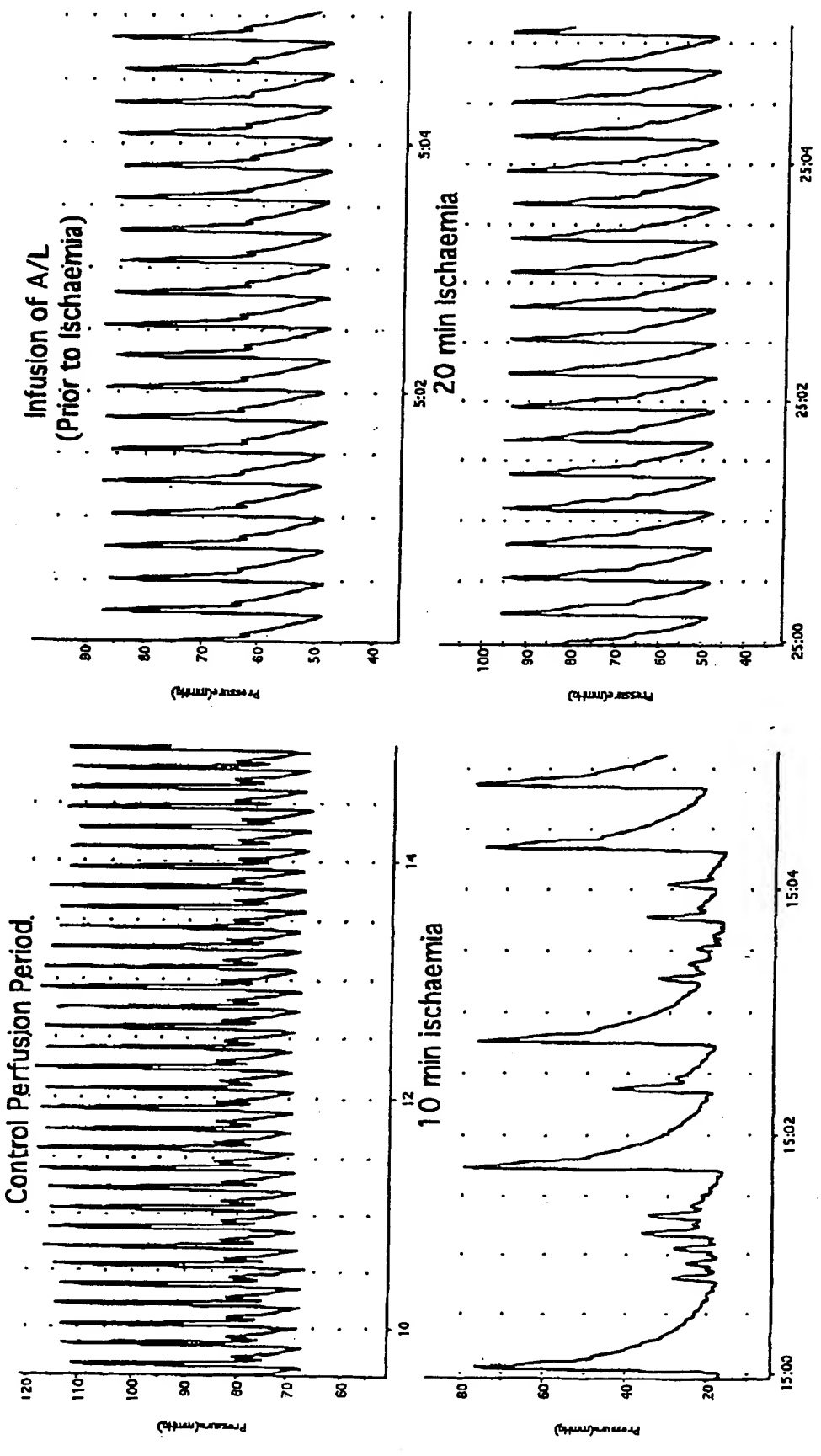
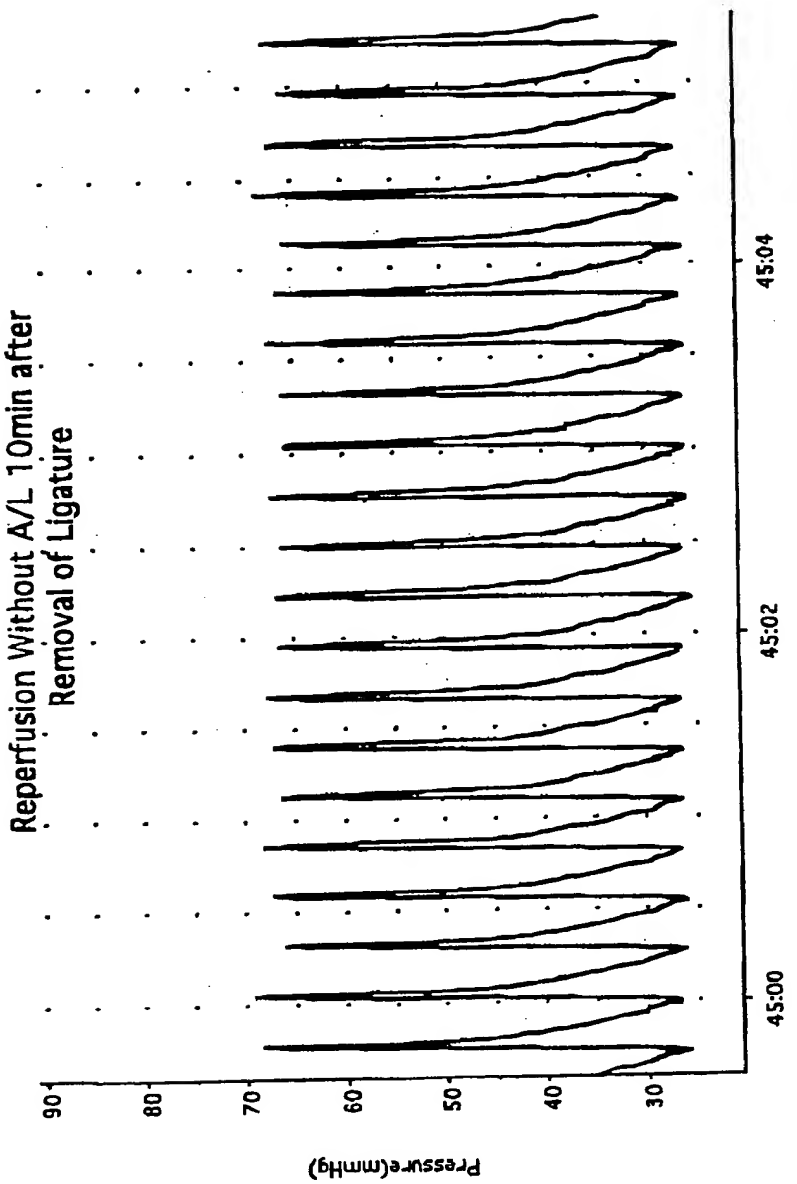

Infusion of A/L
(Prior to Ischaemia)
20 min Ischaemia
Control Perfusion Period
10 min ischaemia

Figure 7


Reperfusion Without A/L 10min after
Removal of Ligature
Pressure(mmHg)
90
80
70
60
50
40
30
45:00
45:02
45:04